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Description of the larva of *Isoperla orobica* Ravizza, 1975 (Plecoptera, Perlodidae), with notes on ecology and distribution in Switzerland

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Abstract

The larva of *Isoperla orobica* Ravizza, 1975, an endemic Alpine species, is described with information about the distribution and ecology in Switzerland. New barcodes were generated to support the association between life stages. The species is associated with springs and spring brooklets. In Switzerland, the species occurs almost exclusively on the southern slopes of the Alps in the cantons Valais, Ticino and Grisons.

Key Words

Stoneflies, morphology, COI, Alps, endemism, taxonomy, larval description

Introduction

Isoperla Banks, 1906 is the most species-rich genus among the stonefly family Perlodidae, with at least 190 valid species recognized from the Holarctic and Oriental Realms (DeWalt et al. 2023). Sixty-three species are reported in Europe, with most of them in the Mediterranean region (DeWalt et al. 2023). Eight species are reported from Switzerland (Roesti 2021), belonging to five different species groups: *acicularis*: *Isoperla carbonaria* (Aubert, 1953), *I. lugens* (Klapálek, 1923); *grammatica*: *I. grammatica* (Poda, 1761), *I. felderorum* (Roesti, 2021); *obscura*: *I. obscura* (Zetterstedt, 1840); *oxylepis*: *I. orobica* (Ravizza, 1975), *I. oxylepis* (Despax, 1936); *rivulorum*: *I. rivulorum* (Pictet, 1841) (Muranyi 2011). *Isoperla lugens* and *I. orobica* are endemic species to the Alps (Gtraf et al. 2009). In Switzerland, all but two species (*I. orobica*, *I. felderorum*) are well known also in terms of their larval stage (Lubini et al. 2012). This study aims to newly describe the larva of *I. orobica*, which is currently only known in the adult stage. This will enhance our knowledge of the distribution of *Isoperla* species in the context of the Swiss monitoring project of aquatic insects. Furthermore, the distribution and ecology of the species in the Alps are discussed.

Materials and methods

All specimens were caught by hand, the larvae by kicknet sampling or by picking specimens directly from the substrate, and the adults by sweep net or "Japanese umbrella" (beating sheet). The specimens were stored in 80% ethanol and deposited in the collection of the Muséum cantonal des sciences naturelles, Lausanne, Switzerland. Mouthparts, abdominal tergites and cerci were mounted on slides and photographed. To distinguish *I. orobica* from sympatric species such as *I. carbonaria* and *I. rivulorum*, we examined comparative material, refering to Lubini et al. (2012) regarding most morphological

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characters. The terminology of the maxilla follows Sandberg (2011). Local and altitudinal distribution as well as the phenological data were provided by the National Center for Wildlife Data and Information in Switzerland (https://infofauna.ch), the Italian distribution by R. Fochetti (Tuscia University).

To provide additional evidence supporting the association between larvae and adults, we conducted genetic sequencing of a 658 bp fragment from the mitochondrial gene cytochrome oxidase subunit 1 (COI hereafter), which corresponds to the standard animal barcode region. We conducted this sequencing on three specimens of *I. orobica*, consisting of two larvae and one adult male (Table 1), using the non-destructive DNA extraction procedure described in Vuataz et al. (2011). The Polymerase Chain Reaction (PCR), purification and sequencing steps were conducted according to the methodology described in El Alami et al. (2022). Forward and reverse sequencing reads were assembled and edited in Codon Code Aligner 10.0.2 (Codon-Code Corporation, Dedham, MA), and aligned in Jalview 2.11.2.7 (Waterhouse et al. 2009). To tentatively augment our dataset, we searched for COI sequences associated with *I. orobica* in the GenBank and Barcode of Life Data System (BOLD) databases as of September 21, 2023, but no additional sequences were available.

Material examined

Isoperla orobica: Switzerland: 6 larvae, spring brooklet, tributary to river Taferna (VS), 46°15'53"N, 8°02'09"E, 1580 m, 6.VII.2017, leg. Verena Lubini, 1 larva DNA, GBIFCH00658280; 8 larvae, same locality, same date, leg. Remo Wüthrich; 4 larvae, same locality, 7.VII.2022, leg. Verena Lubini; 4 larvae, tributary to Fiume Bello, Alpe Rompiago (TI), 46°05'25"N, 8°59'44"E, 1131 m, 28.VI.2018, leg. Verena Lubini; 4 larvae, tributary to Riale Vadina, Alpe di Neggia (TI), 46°06'46"N, 8°50'55"E, 1289 m, 27.VI.2018, leg. Remo Wüthrich; 1 larva, 3 ♂, tributary to Riale Vadina, Rotiscia (TI), 46°07'05"N, 8°51'01"E, 996 m, 27.VI.2018, leg. Remo Wüthrich, 1 ♂ DNA, GBIFCH00658286; 6 larvae, same locality, 29.VI.2022, leg. Verena Lubini and Viviane Uhlmann; 1 larva DNA, GBIFCH00658284, tributary to Riale Vadina, Alpe di Neggia (TI), 46°06'47"N, 8°51'11"E, 1250 m, 29.VI.2022, leg. Verena Lubini; spring brooklet, tributary to river Laggina, Alte Staful (VS), 46°09'30"N, 8°03'24"E, 1550 m, 7.VII.2017, leg. Remo Wüthrich. Italy: 1 larva, 1 ♂, spring brooklet, Valchiusella, 45°32'4"N, 7°40'48"E, 1200 m, 6.VIII.2016, leg. Verena Lubini.

Isoperla carbonaria: Switzerland: 3 larvae, Val Giona, Büèe (TI), 46°06'18"N, 8°50'18"E, 1135m, 5.VI.2000, leg. Verena Lubini; 1 larva, Val Muggio (TI), 45°53'53"N, 9°02'32"E, 544 m, 5.VI.2000, leg. Verena Lubini; 1 larva, Valle Verzasca, Gerra (TI), 46°19'37"N, 8°48'08"E, 819 m, 14.IV.2023, leg. Paola Gandolfi.

Isoperla rivulorum: Switzerland: 1 larva, Alp Prüma, Val Roseg (GR), 46°27'29"N, 9°52'52"E, 1913 m, 27.III.2000, leg. Verena Lubini; 1 larva, spring brooklet, tributary to Rigiaa, Gruonholz (SZ), 47°02'25"N, 8°30'22"E, 1176 m, 19.VI.2022, leg. Emil Birnstiel; 1 larva, Munt San Franzesch (GR), 46°22'45"N, 10°06'03"E, 2150 m, 27.IV.2001, leg. Verena Lubini; 1 larva, river Landquart, Grüsch (GR), 46°58'27"N, 9°38'02"E, 593 m, 30.III.1993, leg. Verena Lubini.

Results

The alignment of the three COI sequences was 100% complete, with no missing data, and all sequences were identical, except for a single site that was ambiguous in the GBIFCH00658280 sequence (Table 1). These findings strongly support the conspecificity of the sequenced larvae and adult. As of September 21, 2023, a BLAST search on our sequences showed the closest match to be a sequence with a 94.1% similarity, originating from a specimen collected in Croatia and identified as *I. rivulorum* (Hlebec et al. 2021), while all other results were less than 94% similar, confirming that our sequences are the first *I. orobica* barcodes to be publicly deposited.

Description of the mature larva

Body length 8.9–11.3 mm (n = 14). General colour light brown, abdomen notably darker than head and thorax (Fig. 1A, B, 6C), ventral coloration pale (Fig. 1D, E). Pilosity typical of the genus, thoracic segments covered by dark brown clothing hairs and blunt marginal spines.

Head light brown with indistinct pale markings; defined pale interocellar area lacking; frontoclypeus anterior to M-line pale; sometimes two darker sublateral markings at anterior margin of frontoclypeus; pale sublateral occipital spots posterior to epicranial suture; head trapezoid shaped with frontal area slightly elongated (Fig. 2A). Scape, pedicel and following antennomeres pale; palpi and mouthparts pale (Fig. 2B).

Lacinia bidentate, sub-quadrate shaped with only slightly rounded inner margin; subapical tooth slightly longer than half of the length of apical tooth; 1 group A submar-

Table 1. Newly sequenced *Isoperla orobica* specimens from Switzerland, including collection details and GenBank accession numbers.

Specimen catalogue nb	Stage	GPS Coordinates	Altitude	Date	Collector	GenBank ID
GBIFCH00658280	Larva	46°15'53"N, 8°02'09"E	1580 m	6.VII.2017	V. Lubini	OR600533
GBIFCH00658284	Larva	46°06'47"N, 8°51'11"E	1250 m	29.VI.2022	V. Lubini	OR600534
GBIFCH00658286	Adult male	46°07'05"N, 8°51'01"E	996 m	27.VI.2018	R. Wüthrich	OR600535

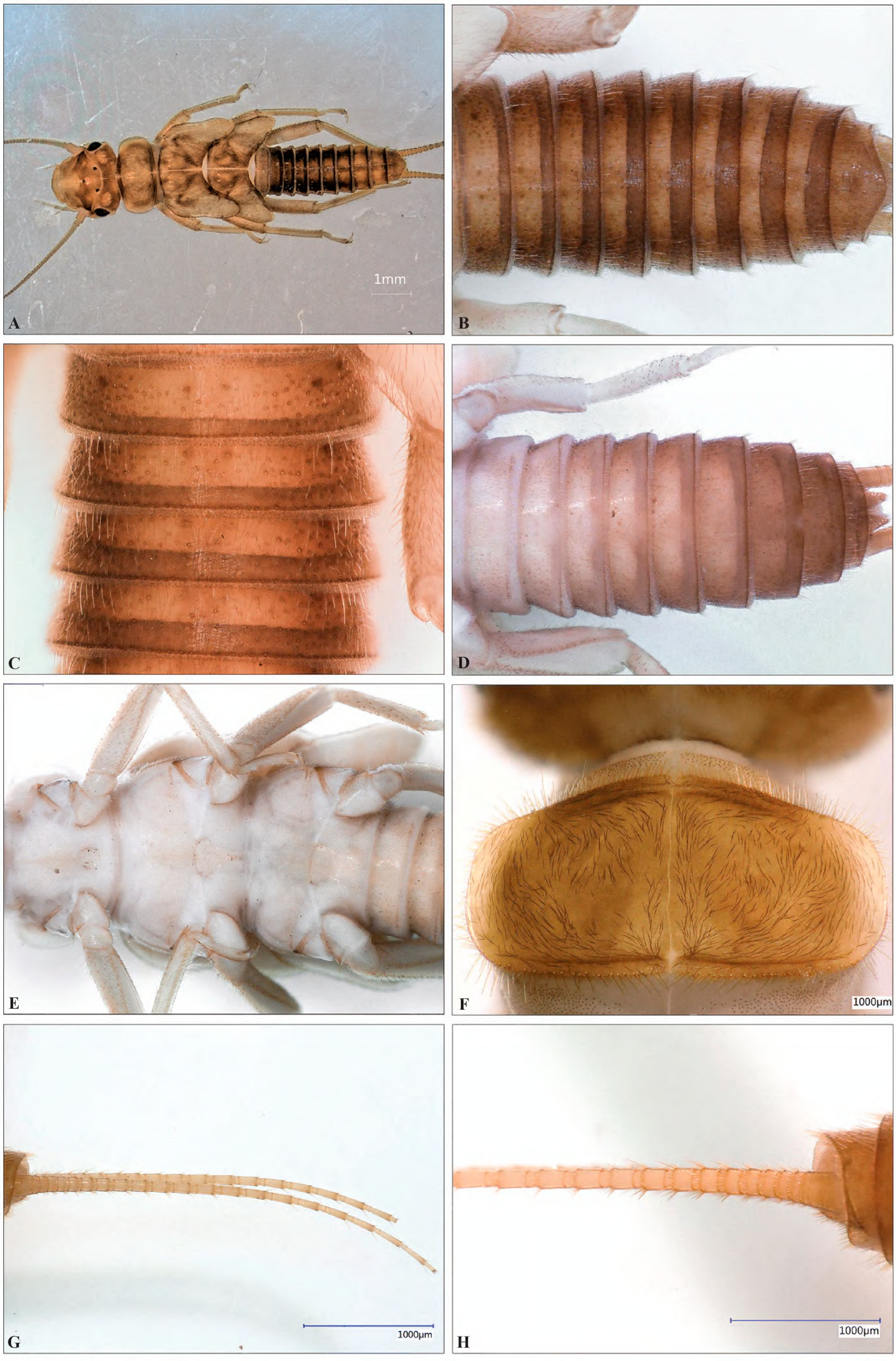

Figure 1. *Isoperla orobica*, larval morphology. **A.** Habitus dorsal; **B.** Abdomen dorsal; **C.** Tergite 1–4; **D.** Abdomen ventral; **E.** Furcasternites; **F.** Pronotum; **G.** Left cercus lateral; **H.** Right cercus detail lateral. (Photos: Pia Teufl, Christoph Novotny).

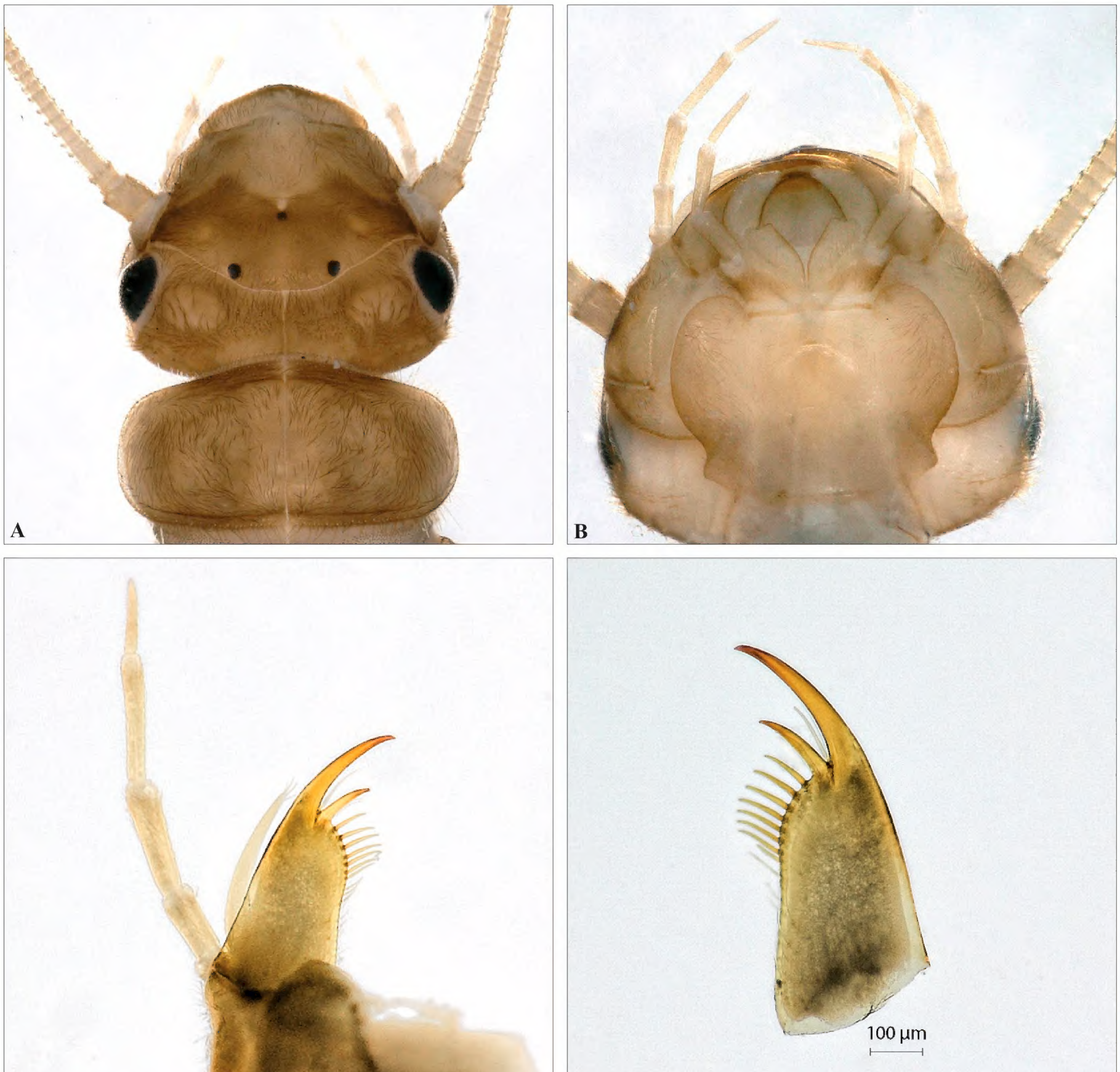

Figure 2. *Isoperla orobica*, larval morphology. **A.** Head and pronotum dorsal; **B.** Head ventral; **C.** Left lacinia and galea dorsal view; **D.** Right lacinia dorsal view; **E.** Right mandible dorsal view; **F.** Right mandible ventral view (Photos: Pia Teufl, Christoph Novotny).

ginal setae present, below apical tooth and adjacent to subapical tooth; 1 thin marginal seta below apical tooth and 1 thin marginal seta proximal to subapical tooth; 6–10 stout marginal setae, space between setae continuously decreasing towards base, almost all stout marginal setae of equal length except for the most proximal setae, which are shorter; sparse row of thin marginal setae (9–12) along proximal half of inner lacinial margin (Fig. 2C, D); scattered, sparse ventral surface setae on inner basal palm. Galea with four apical setae; inner and outer margin with sparse setae; galea reaching base of apical tooth. Mandible with one incisor and three molars, molar brush dense and long; dense seate dorsally and ventrally at inner margin; small area of setae laterally at the base of mandibles (Fig. 2E, F).

Pronotum light brown, lateral margins of disc lighter in colour; anterior and posterior margin of pronotum slightly darker; half as long as wide (ratio 1:2); spines on protonal margin distinct; longest spines as long as one fifth of the pronotal width with shorter spines in between (Fig. 1F).

Mesonotum and metanotum with indistinct darker markings; wingpads pale; outer margin of wingpads slightly concave (Fig. 1A). Thoracic sternites pale; furcasternites and furcal pits hardly contrasting (Fig. 1E).

Abdominal terga mostly brown with variable dark median marking; first tergite lighter; posterior margin of tergites 1–10 with long blunt spines, longest spines longer than half of tergal lengths (Fig. 1C). Sternites pale, distal segments slightly darker.

Legs light brown; dense row of acute spines covering dorsal margin of femur; spines short on proximal third and longer (length: half of femur width) on two distal thirds of femur; long silky setal fringe (almost as long as femur width) covering dorsal margin of femur (Fig. 3B); spines and setae on surface of femur (Fig. 3A).

Double row of dense spines (length: one third of tibia width) on dorsal margin of tibia (Fig. 3C) with longer ones towards distal third; dorsal side of tibia with sparse and long setal fringe (length: as long as tibia width). Tarsi with sparse setal fringe on dorsal margin (Fig. 3D). All claws with one basal tooth each.

Cercus pale yellow with a thin brown ring on proximal 1/4 of cercomeres; cercomeres cylindrical; medial cercomeres twice as long as wide. Cercal apical fringes dense, circumferential setae on medial cercomeres approximately one fourth of segment length, dorsomedial and ventromedial setae longer (length: more than half of segment length); cercus without dorsal setal fringe (Fig. 1G, H). Paraproct blunt with widely rounded apex.

Figure 3. *Isoperla orobica*, larval morphology. **A.** Front left femur; **B.** Front left femur, dorsal margin; **C.** Front left tibia; **D.** Front left tarsus (Photos: Pia Teufl, Christoph Novotny).

Distribution and ecology

Isoperla orobica is an endemic rheophilic species from the western and central Alps (Graf et al. 2009; Fochetti 2020), distributed only in northern Italy (Consiglio 1979; Ravizza 2023) and Switzerland (Fig. 4). In Switzerland, the species is predominantly observed along the southern slopes of the Alps, with its current distribution primarily centered on the Simplon Pass, as well as in certain mountainous regions within the canton Ticino (Val Colla, Valle di Vira, Val Serdena) and the canton Grisons (Val Mesocco) (https://infofauna.ch). Apart from the southern slopes of the Alps there is only one location in the central Alps, which is situated in the catchment of the river Rhone in the canton Valais, Switzerland.

The larvae were found in springs, spring brooklets and in brooks of the epirhithral zone mainly between 900 m and 1500 m, rarely above 2000 m. The substrate varies from organic to coarse stony bottoms in moderate to steep slopes (Fig. 5). The flight periods of the adult insects extend from late June to October at altitudes ranging from 900 m to 2030 m. The accompanying fauna consists of *Dictyogenus fontium* (Ris, 1896), *Chloroperla susemicheli* (Zwick, 1967), *Siphonoperla italica* (Aubert, 1953), *Leuctra insubrica* (Aubert, 1949), *L. armata* (Kempny, 1899), *L. braueri* (Kempny, 1898), *L. caprai* (Festa, 1939), *L. dolasilla* (Consiglio, 1955), *L. schmidi*

Figure 4. Present distribution of *I. orobica* in Switzerland and Italy. Only one location is in the drainage area of the river Rhone (red star), all other locations (red squares) are in the drainage basin of the river Po. The Swiss locations represent areas of 5×5 km with a total of 30 sampling stations (https://infofauna.ch).

Figure 5. Habitat of *I. orobica* in Switzerland, upstream views. **A, B.** Spring brook, tributary to river Taferna (Rhone basin, canton Valais); **C.** River Vadina, tributary to Lake Maggiore (canton Ticino); **D.** Brook in Simplon pass, near Stalde (canton Valais) (Photos: Verena Lubini).

(Aubert, 1946), *L. vinconi* (Ravizza & Ravizza Dematteis, 1993), *Nemoura sinuata* (Ris, 1902) and *Protonemura lateralis* (Pictet, 1836).

Diagnosis

For comparison between *I. orobica* and the two sympatric species *I. rivulorum* and *I. carbonaria*, body coloration patterns and characteristics of the maxillae were considered (Table 2). In terms of coloration, *I. orobica* differs from both other species (Fig. 6, Lubini et al. 2012) in having a uniformly brown colour and lacking a pale interocellar area on the head.

The lacinia of all three species is bidentate and generally sub-quadrate, but differences in the number of thin marginal setae on the lacinia and pilosity of the galea can aid in distinguishing these taxa. *Isoperla orobica* has 9–12 thin marginal setae compared to 25–35 in *I. rivulorum* (Table 2, Fig. 7). Additionally, *I. orobica* has setae on both the inner and outer margins of the galea; whereas *I. carbonaria* only has setae on the outer margin. Furthermore, *I. orobica* lacks the dense setal fringes on the tibiae, as is the case in the other two species.

Table 2. Verification table of morphological characters for *Isoperla orobica*, *I. rivulorum* and *I. carbonaria* (SMS = stout marginal setae; TMS = thin marginal setae).

Species	Body color	Interocellar area	Lacinia SMS	Lacinia TMS	Galea setae
Isoperla orobica	uniform	indistinct	6–10	9–12	inner & outer
Isoperla rivulorum	contrasting	distinct	7–8	25–35	inner & outer
Isoperla carbonaria	contrasting	distinct	7–8	10–16	outer

Figure 6. Larval habitus. **A.** *I. carbonaria*; **B.** *I. rivulorum*; **C.** *I. orobica* (Photos: Verena Lubini).

Figure 7. Lacinial morphology. **A.** *I. carbonaria*; **B.** *I. rivulorum* (Photos: Laurent Vuataz).

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